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AN ANTISERUM AGAINST TRANSFORMING GROWTH FACTOR β SUPPRESSES EXPERIMENTAL GLOMERULONEPHRITIS

Wayne A. Border[1], Seiya Okuda[1], Kathleen C. Flanders[2], Lucia R. Languino[3], Michael B. Sporn[2] and Erkki Ruoslahti[3]

[1]Division of Nephrology, 50 No. Medical Drive, Salt Lake City, UT 84132
[2]Laboratory of Chemoprevention, National Cancer Institute, NIH Bethesda, MD 20892
[3]La Jolla Cancer Research Foundation, 10901 N. Torrey Pines Rd., La Jolla, CA 92037

Exhibit E

ABSTRACT

Experimental glomerulonephritis induced in rats by injection of anti-Thy-1 antiserum is associated with increased transforming growth factor β (TGFβ) activity and enhanced production of glomerular extracellular matrix. Administration of anti-TGFβ after the induction of the glomerular disease suppressed the elevated extracellular matrix production and attenuated other manifestations of the disease. These results demonstrate a role for TGFβ in the pathogenesis of the experimental disease and suggest a new form of therapy for glomerulonephritis with anti-TGFβ.

Nephritis was induced in rats with a single injection of anti-Thy-1 serum and the rats were then treated with either injections of anti-TGFβ or normal rabbit serum as a control. Ten animals were used in each group in two different experiments. Figure 1 shows a comparison of representative microscopic fields from kidneys of the two treated and control animals. The glomeruli have expanded less and contain less extracellular matrix in the anti-TGFβ-treated group than in the normal rabbit serum controls. Biochemical analysis showed that proteoglycan production by mesangial cells, which is high in the cells from the injured kidneys (4), was suppressed to a near normal rate by anti-TGFβ (Figure 2). Scanning of the gel bands in Figure 2 and from other similar experiments indicated that the suppression of this measure of the disease process was about 60%. Examination of proteinuria also indicated a milder course of disease in the rats treated with anti-TGFβ. All control rats had severe proteinuria (Xμg/ml) between X and Y days after the injection, whereas only X of the treated rats showed proteinuria (X-Y μg/ml). These results show that the disease was substantially attenuated by the anti-TGFβ treatment.

To gain information on the mechanism of the anti-TGFβ effect, we examined the level of TGFβ mRNA in the kidneys of the treated and control rats. TGFβ can stimulate its own production (11). Therefore, an agent that inhibits the activity of TGFβ may also reduce its synthesis. The mRNA analysis revealed elevated levels of TGFβ mRNA in the nephritis rats (Figure 3A) and showed that this level was several fold lower in the anti-TGFβ treated animals (Figure 3B). These results suggest that the antibody had interrupted an autostimulatory loop of TGFβ induction.

Our findings establish a central role for TGFβ in the pathogenesis of acute experimental glomerulonephritis. It is well known that TGFβ can greatly stimulate the production of extracellular matrix components by various kinds of cells (6,12), including the production of the two proteoglycans we have used as markers of TGFβ activity in this and earlier studies (4,7). Because of this activity, TGFβ has for some time been suspected to play a role in fibrotic diseases resulting from various chronic disease processes (14). A surprise in our experiments was the involvement of TGFβ in acute glomerular disease and that the manifestations of this disease could be so effectively suppressed with anti-TGFβ treatment. The explanation may be the extremely strong stimulation of extracellular matrix synthesis, manifested as an almost 50-fold increase of the proteoglycans biglycan and decorin in our model, by TGFβ. The ability of TGFβ to suppress the expression of proteases and stimulate the expression of protease inhibitors (15) may also contribute to the accumulation of extracellular matrix. The resulting drastic changes in matrix production may sufficiently distort the glomerular architecture to account for the proteinuria and microscopic changes.

Our results not only contribute to the understanding of the pathogenesis of nephritis, they point to a new direction in its therapy. The experimental model we have used here is considered a good approximation of the human disease (3). The impressive suppression of the experimental disease achieved with the anti-TGFβ treatment, therefore, encourages one to expect similar potential benefits in human glomerulonephritis and perhaps in other diseases as well where fibrosis is a factor.

FIGURE LEGENDS

Figure 1. Micrographs showing the enlargement of glomeruli in nephritic kidneys. Kidneys from rats made nephritic by an injection of anti-Thy-1 antiserum and were examined on the same day (A and D), and four days (B and E) or seven (F) days after the injection. Panels A, B, C are from rats that received normal rabbit serum injections three successive days, starting on the day of the anti-Thy-1 injection and panels D, E and F are from animals that received rabbit anti-TGFβ under a similar regimen. Hematoxylin-eosin staining. Bar X μm.

Methods: Rats, origin of anti-Thy-1. The anti-TGFβ antiserum was prepared against a synthetic peptide from residues 78-109 of the human mature TGFβ. Antisera raised against the same peptide, whose terminal cysteine residues were disulfide-linked, have previously been shown to inhibit the binding of TGFβ to its receptors (9). The peptide was synthesized in a Applied Biosystems solid phase peptide synthesizer and purified by HPLC. A rabbit was immunized with 2 mg per injection of the peptide mixed with 0.5 mg of methylated BSA (10) and emulsified in Freund's complete adjuvant. The injections were generally given four weeks apart and the rabbit was bled approximately a week after the second and every successive, injection. The bleedings used in this work had a titer (50% binding) of 1:30,000-radio immunoassay, bound to TGFβ in immunoblots and inhibited the induction of proteoglycan synthesis caused by TGFβ in cultured mesangial cells (not shown).

Figure 2. Proteoglycan synthesis by glomeruli from nephritic rats treated with TGFβ. Glomeruli were isolated 4 and 7 days after an anti-Thy-1 injection that

has been followed by treatments similar to those described in the legend of Figure 1, and placed in culture. Proteoglycan synthesis was examined by labeling the cultures with $^{35}SO_4$ followed by analysis of the secreted products by SDS-PAGE and autoradiography as described (4,7). NRS, nephritic rats treated with normal rabbit serum αTGFβ, nephritic rats treated with rabbit anti-TGFβ. The control lane shows proteoglycan production in glomeruli from a normal kidney and the first lane contains molecular weight markers.

Figure 3. Northern blotting of TGFβ mRNA in glomeruli isolated from the kidneys of nephritic rats.

Methods: